Journal of Animal and Veterinary Advances 12 (17): 1376-1382, 2013
ISSN: 1680-5593
© Medwell Journals, 2013

# Network-Based Insight Analysis of Drugs of China Food and Drug Administration for Potential New Multi-Target Drug Discovery

Nan Zhou, Jin-Chun Zhang, Yong-Xi Liu, Yang Yu,
Yuan Deng, Ling Feng, Wei Qi, Chuan-Fang Wu and Jin-Ku Bao
Key Laboratory of Bio-Resources, School of Life Sciences,
Ministry of Education, Sichuan University, 610064 Chengdu, China

**Abstract:** Developing multi-target drugs to obtain potentially innovative medicines has become a trend in the treatment of multifactorial diseases. The open-access resources are used by computational biologists to uncover relationships among various datasets for further drug discovery. In this study, researchers systematically analyzed approved retail drugs of China Food and Drug Administration (CFDA) in terms of biological interactions networks and found that CFDA-approved drugs had significant multi-target properties. To determine the features of these drugs and understand their indication on multi-target drug design, researchers computationally built a bipartite graph composed of drugs and target proteins linked by drug-target binary associations. Furthermore, researchers chose 19 drugs whose target numbers were ≥15 and then integrated human Protein-Protein Interactions (PPIs) datasets from DIP, IntAct, BioGRID, MINT and HPRD to generate a human PPIs network to analyze targets of these drugs. Graph theory analysis identified significant nodes including five multi-target drugs and eight drug targets which indicated that some of the CFDA-approved drugs were potentially valuable for the future development of multi-target drugs.

**Key words:** Drug-target, computationally, bipartite graph, graph theory, CFDA

## INTRODUCTION

Cause of the goal of drug discovery is to design exquisitely selective ligands against a single target (Cheng *et al*., 2012), the rate of new chemical entities transferred to therapeutic agents decreased in recent years (Hopkins, 2008; Cheng *et al*., 2012). Fortunately, as more and more scientists realized that many existing drugs possess an inherently multi-target characteristics, the traditional view that a drug selectively interacts with a specific protein target and the one gene, one drug and one disease paradigm have been challenged (Roth *et al*., 2004; Csermely *et al*., 2005; Hopkins, 2008). For instance, Celecoxib (Celebrex), a selective cyclooxygenase-2 non-steroidal anti-inflammatory drug has been identified to target two additional enzymes (namely, carbonic anhydrase II and 5-lipoxygenase) (Weber *et al*., 2004; Sud'ina *et al*., 2008). Additionally, the serotonin and serotonergic drugs are now believed to not only bind to G Protein-Coupled Receptors (GPCRs) but also to an ion channel (5-$HT_3$) (Kroeze *et al*., 2002; Roth *et al*., 2004). Indeed, such polypharmacological features of drugs make us realized that multi-target drugs would more effective and practical than single hits (Csermely *et al*., 2005). Furthermore, the introduction of anticancer drugs like Imatinib (Gleevec) and Sunitinib (Sutent) and non-selective drugs for mood disorders and schizophrenia support that multi-target drugs can play vital roles in the treatment of complex diseases (Roth *et al*., 2004). Traditional medical treatments often use multi-component extracts from natural products (Csermely *et al*., 2005) and many CFDA-approved drugs are transformed from traditional Chinese medicines which could hit multiple targets and exert synergistic therapeutic efficacy (Raffa *et al*., 2003; Tao *et al*., 2013).

Traditional Chinese medicine has been recognized as a complementary and alternative medicine in Western countries and the mixed application of Western medicine received much attention (Kairuz *et al*., 2007; Xu, 2011). Ho (1995) introduced cocktail therapy to treat AIDS and verified the effectiveness of mixed drugs with advantages of enhanced potions, reduced toxicity complementary onset time and extension efficacy. In addition, some compound drugs have been approved by FDA because of their effectiveness, such as Benicar HCT (combination of hydrochlorothiazide and olmesartan), Aggrenox (combination of aspirin and dipyridamole) and Glucovance (combination of glyburide and metformin) (Forbes, 1998; Marre and Allavoine, 2000; Chrysant *et al*., 2004).

**Corresponding Author:** Jin-Ku Bao, Key Laboratory of Bio-Resources, School of Life Sciences, Ministry of Education, Sichuan University, 610064 Chengdu, China

The basis of drug discovery and design is the interaction between the drug and its target (drug-target interaction). Therefore, the drug-target interactions network is generated to help finding new targets and new effective drugs at lower cost, detecting cross-pharmacology relationships among targets and designing multi-target drugs that interact only with effective targets (Yamanishi *et al*., 2008; Lee *et al*., 2009; Vogt and Mestres, 2010). Network structure is crucial for drug target identification and topological analysis of network contributes largely to the initial understanding of multi-target drug actions (Csermely *et al*., 2005). Based on the systematic analysis of biological networks, the integration of relevant data can provide a more global view on drug-target relations. In order to figure out whether the CFDA-approved drugs could make contribution to the development of novel multi-target drugs by analyzing the drug-target network of CFDA, researchers retrieved datasets from databases and analyzed the relationships between drugs and their targets using statistical methods; the drug-target bipartite network and human PPIs network were constructed for topological analysis of 19 multi-target drugs and their targets; CFDA-approved multi-target drugs and their corresponding targets were analyzed in the context of biological networks and the potentiality of these drugs in the further research in novel multi-target drug creation were discussed.

## MATERIALS AND METHODS

**Database of drug-target interaction:** The drug list with 7209 approved drugs was downloaded from the website of CFDA (http://www.sfda.gov.cn) and their corresponding targets were obtained from the DrugBank (Knox *et al*., 2011) database at http://www.drugbank.ca. As of November 3, 2012, DrugBank contained 3291 human target proteins. Therefore, the two datasets can be used to construct the drug-target interactions network.

**Database of human protein-protein interactions:** The human PPIs datasets were downloaded from 5 databases as follows: DIP (Salwinski *et al*., 2004), BioGRID (Stark *et al*., 2011), IntAct (Kerrien *et al*., 2012), HPRD (Prasad *et al*., 2009) and MINT. And then the datasets were initially processed by integrating all the various datasets and eliminating the duplicates. Finally, the processed dataset including 128896 interactions among 16309 human proteins was used to construct the PPIs network.

**Construction of biological networks:** In the research, all biological networks were generated and displayed by Cytoscape 2.8.3 (Smoot *et al*., 2011). Firstly, researchers constructed a Drug-Target network (D-T network) with the dataset of drug-target interactions. Thus, researchers applied a one-mode projection to the D-T network resulting in two monopartite networks. One was Drug-Drug network (D-D network) constructed by linking two drugs sharing same targets, the other was Target-Target network (T-T network) constructed by linking two targets if they shared same drugs (Barabasi and Oltvai, 2004).

Secondly, the PPIs network was constructed by the human PPIs dataset where the nodes represented proteins and edges between the nodes displayed the interactions of different proteins. Cause of the human global PPIs network was too large to analyze, researchers constructed a sub-PPIs network by linking 217 target proteins of 19 multi-target drugs with other human proteins for analyzing effectively.

**The topological properties:** Notably, the key feature of many large networks is the vertex connectivity followed a scale-free power-law distribution following $P(k) \sim k^{-g}$ that is to say it is a common property of all interacting biological networks (Barabasi and Albert, 1999; Ruffner *et al*., 2007; Chautard *et al*., 2009). Quantitative description of these networks benefits the characterization of various biological systems. In the research, researchers used three predefined properties (degree, average degree and network density) from graph theory to reveal the complexity of interactions networks (D-T, D-D, T-T, PPIs and sub-PPIs). Degree (ki) is the most elementary characteristic of a node and it represents the number of edges connecting to it. Average degree (k) is the average edges per node. The density shows how densely the network is populated with edges (self-loops and duplicated edges are ignored) (Dong and Horvath, 2007) and it has a value between 0 and 1. A network which contains no edges and solely isolated nodes has a density of 0. In contrast, the density of a clique is 1 (Assenov *et al*., 2008).

## RESULTS AND DISCUSSION

**Statistic analysis:** Researchers downloaded the list of drugs from CFDA and obtained the corresponding protein targets from DrugBank dataset. Then, researchers analyzed drugs with respect to the number of their targets (Fig. 1a) and proteins according to the number of drugs targeting them (Fig. 1b). Researchers can infer from Fig. 1a that some drugs had more than one protein targets while others targeted only one protein in current application. According to Fig. 1b, though lots of targets were targeted by only one drug, there were some proteins targeted by

more. Thus, the overlapped target proteins indicated a current shortcoming of drug discovery that was using the already known targets. Among FDA-approved drugs, one drug targeted at most 14 proteins (Yildirim *et al*., 2007). However, there were 19 CFDA-approved drugs had >14 targets. There were 417 unique interactions between these drugs and 217 corresponding target proteins which meant every drug was currently acknowledged to interact with 12 targets.

**D-T network analysis of the 19 multi-target drugs:** The visualization of the D-T network provides an important survey method for the current drug discovery status. If amounts of drugs only targeted few proteins, the network would have contained isolated nodes with few or even no edges between them. Instead, the network would have many interactions among different drugs.

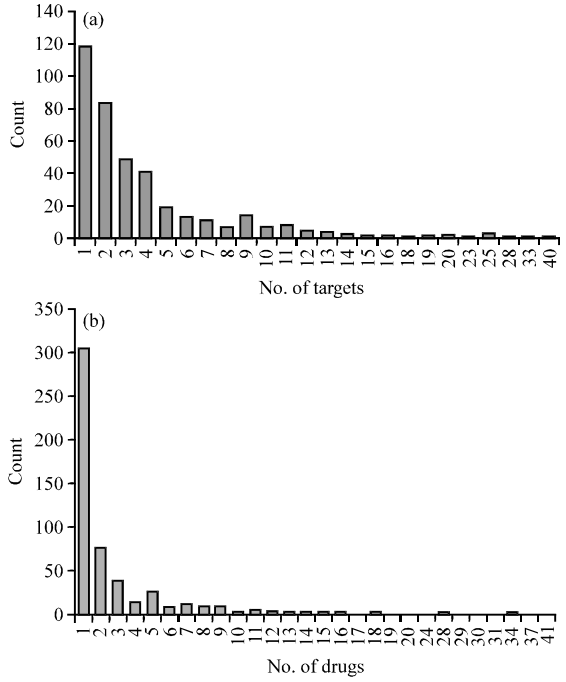


Fig. 1: Distributions of drugs and drug target proteins; a) Distribution of drugs with respect to the number of their target proteins. The CFDA-approved drugs targeted 551 human proteins in total. Most drugs targeted only a few target proteins but some had many target proteins for example, reduced glutathione for Injection had 40 target proteins; b) Distribution of target proteins with respect to the number of drugs a protein was targeted. The most-targeted target proteins were the α-1A Adrenergic receptor (ADA1A) (41 drugs), the α-2A adrenergic receptor (ADA2A) (37 drugs), the Histamine H1 Receptor (HRH1) (targeted by 34 drugs) and the β-2 Adrenergic receptor (ADRB2) (34 drugs)

According to the research, the D-T network (Fig. 2) had 1861 unique interactions between 415 drugs and 551 human proteins. Every multi-target drug targeted >14 proteins and 217 targets in total which accounted for 39% of all the 551 protein targets. The topology of the D-T network with a well-organized modular structure reflected the cluster of drugs and targets. In the D-T network, the average degree of 19 drugs was 21.947, much higher than 4.484 of all 415 drugs. Several drugs were located in the giant interconnected component in the network (red rectangle marked) while some other formed independent clusters. It indicated that among 217 target proteins, some had been studied thoroughly and utilized fully while others were not which should call for the attention to discover novel drugs. Even though researchers are not fully aware of the presence of multiple targets for certain drugs, it shall target whatever proteins it should target in the human body to take effect. With respect to drugs formed independent clusters, being apart from the big clusters in the D-T network, they may be used to treat some highly specific diseases. A combination drug named glutamic acid, alanine acid and glycine acid tabletes was used to treat the prostate

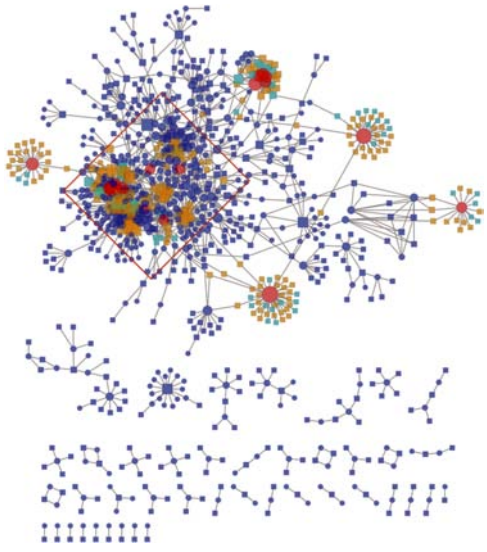

Fig. 2: The drug-target network. Circles and rectangles represented drugs and target proteins, respectively. Nodes in red were the 19 multi-target drugs with >14 target proteins. For all the 217 targets of 19 drugs, orange represented targets in the human PPIs and bright green represented targets not in

hyperplasia caused by the frequency micturition, dysuria and urinary retention disorder, especially suitable for cardiopulmonary function incomplete and too old for surgery.

**D-D network and T-T network analysis of the 19 multi-target drugs:** The D-D network showed the status of different drugs with common target (s). If a lot of drugs are based on the same targets then the D-D network would interconnect tightly. On the contrary, drugs in the D-D network would be distributed. Through such characteristic, researchers can reasonably estimate the utilization of targets of multi-target drugs and analyze the cross-pharmacology relationships between targets, ultimately guide new drug design (Vogt and Mestres, 2010).

Drugs connecting two significant clusters were defined as bridge drug and had special indication. In the D-D network (Fig. 3a), researchers found 5 bridge multi-target drugs, named compound phenobarbital nitrazepam and chlorphenamine maleate tablets, midazolam injection, clonazepam tablets, triazolam tablets and lorazepam tablets. These clinic nerve drugs were used to calm patients with epilepsy. To the knowledge, diseases in the nervous system are so complicated that the target-specific drugs are less curative (Scheffer, 2004). Therefore, these drugs and their targets could be used to develop novel drugs for complex diseases, such as nervous system diseases.

The T-T network provided a complementary, protein-centered view of pharmacological space (Paolini *et al*., 2006). In Fig. 3b, 536 out of 551 target proteins were connected to each other and multi-target drugs were responsible for the high interconnectedness. There were 8 target proteins formed bridges between two clusters to be worthy of consideration. Among them, Glutathione Synthetase (GSHB), Dopamine β-hydroxylase (DOPO) and Glycine Receptor subunit α-1 (GLRA1) involve in diseases of Glutathione Synthetase deficiency (GSS), Dopamine β-hydroxylase deficiency (DBH) and hyperekplexia, hereditary, type 1 (HKPX1). And that their average degree was 7.625, <15.609 of all proteins in PPIs network (Fig. 4a). Namely, these proteins were not hub proteins in human PPIs network and drugs of these targets had little side-effects (Jeong *et al*., 2001).

**Target proteins of 19 multi-target drugs within human PPIs network:** Among the 217 targets of 19 multi-target drugs, 167 existed in the human PPIs network (Fig. 4a) of which a few of them located in the center. According to the centrality-lethality rule, rather than targeting a protein with high degree, multi-target drugs target proteins of

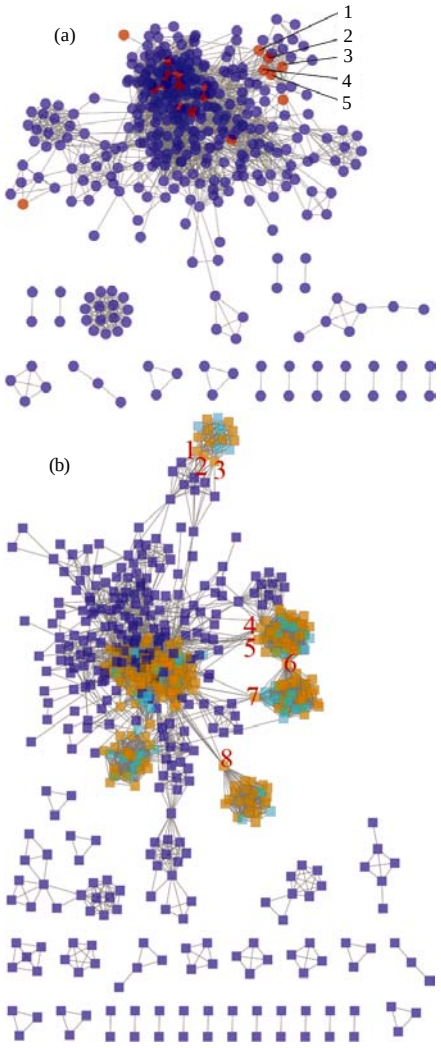


Fig. 3: Two bipartite projection networks of the drug-target network. a) Drug-drug network, two drugs connected by an edge if they shared same target (s). Red nodes indicated drugs that had more than 14 targets. Nodes 1-5 were bridging multi-target drugs; 1: compund phenobarbital N itrazepam and chlorphenamine maleate tablets; 2: midazolam injection; 3: clonazepam tablets; 4: triazolam tablets; 5: lorazepam tablets). b) Target-target network, two target proteins connected by an edge if they were targeted by same drug (s). Nodes colored in orange or bright green represented target proteins that were targeted by the 19 multi-target drugs. In addition, orange nodes were in the human PPIs network but bright green nodes were not. Nodes 1-8 were bridging targets; 1: AL1A1; 2: AL1A2; 3: RET1; 4: GSTP1; 5: LGUL; 6: GSHB; 7: GLRA1; 8: DOPO)

interest but were not the critical components of important pathways to lower side-effect and drug resistance (Csermely *et al*., 2005; Ruffner *et al*., 2007; Kotlyar *et al*., 2012). Voltage-dependent P/Q-type Calcium Channel subunit α-1A (CAC1A), Glutathione S-Transferase κ-1 (GSTK1) and Calmodulin (CALM) had degrees of 94, 92 and 88. And they formed three obvious clusters with network density of 0.035, 0.025 and 0.050, respectively (Fig. 4b). Drugs targeted CAC1A were used to treat chronic stable angina, diarrhea and supraventricular tachycardia. Drugs targeted GSTK1 were used to treat patients undergoing chemotherapy or radiotherapy and all kinds of low oxygen hematic disease, liver disease and drug toxicity, etc. Though these proteins in human PPIs network shared almost the same topological features, their drug numbers were not. There were 14 drugs targeting CALM while only 3 drugs targeted to CAC1A and 1 to GSTK1. Therefore, CAC1A and GSTK1 should also be accounted for future potential drug targets.

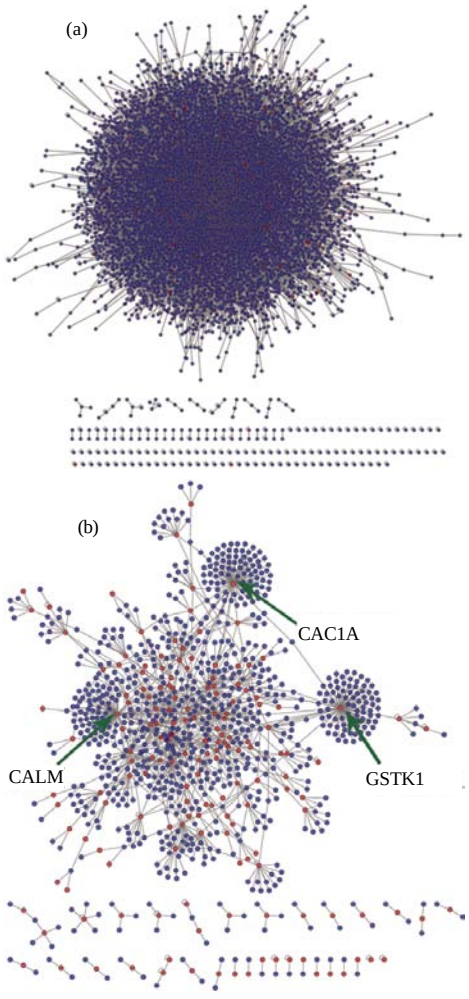


Fig. 4: Interactions between target proteins and other human proteins. Nodes in red were the 167 target proteins of 19 multi-target drugs; a) Target proteins of multi-target drugs within the human PPIs network; b) Sub-PPIs network derived from the human PPIs network showing interactions between the 167 target proteins of 19 multi-target drugs and their interacting human proteins

## CONCLUSION

Building and analyzing networks containing relationships among drugs and targets are one of the latest and pivotal developments in drug discovery (Lee *et al*., 2009). There are several pioneering studies about drug-target networks building. Yildirim *et al*. (2007) built a bipartite graph of drug-protein interactions from FDA-approved drugs and their target proteins to review drug targets in the context of cellular and disease networks. Cases and Mestres (2009) built a ligand-protein interactions map for drug discovery. Their researches were the foundation of drug-target network analysis and might be benefit of cross-pharmacology detection and target identification as well as the multi-target drug development.

In the study, based on the concepts of systems biology, researchers constructed biological networks by integrating datasets of different databases. These networks assisted us to address two questions regarding drug development: What are the features of Chinese commodity drugs? How could these multi-target CFDA-approved drugs enhance future drug development? As the outcome showed, there were 19 drugs targeted >14 targets in CFDA while in FDA, no drug had >14 target proteins (Yildirim *et al*., 2007). These 19 drugs are used to treat complex diseases, such as 9 of them for neurological diseases. In addition, the circumstance of unbalanced development (14 drugs targeted CALM but only 3 drugs targeted CAC1A and one targeted GSTK1) revealed the incomprehensive development of multi-target drugs and need for more attention. Consequently, researchers can use the CFDA-approved multi-target drugs to design novel drugs and find new function of known drugs.

Although, in the research, factors such as data completeness or information loss had impact on the network construction through databases, the integration of relevant data allowed analysis at the network level as well as provided a closer global view on drug-target relations. Therefore, studies using drug-target network analysis and systematic drug-design strategies on multi-target drugs of CFDA would provide further new clues towards possible multi-target drug development.

## ACKNOWLEDGEMENTS

Researchers gratefully thank Dr. Jian Li for providing constructive suggestions, Huailong Xu, Zheng Shi, Na An, Xin Li and Lei Wu for critically reading the manuscript. This research was supported in part by grants from the National Natural Science Foundation of China (No. 81173093, No. 30970643 and No. J1103518) and Special Program for Youth Science and Technology Innovative Research Group of Sichuan Province, China (No. 2011JTD0026). Nan Zhou and Jin-Chun Zhang contributed equally to this research.

## REFERENCES

Assenov, Y., F. Ramirez, S.E. Schelhorn, T. Lengauer and M. Albrecht, 2008. Computing topological parameters of biological networks. Bioinformatics, 24: 282-284.

Barabasi, A.L. and R. Albert, 1999. Emergence of scaling in random networks. Science, 286: 509-512.

Barabasi, A.L. and Z.N. Oltvai, 2004. Network biology: Understanding the cell's functional organization. Nat. Rev. Genet., 5: 101-113.

Cases, M. and J. Mestres, 2009. A chemogenomic approach to drug discovery: Focus on cardiovascular diseases. Drug Discov. Today, 14: 479-485.

Chautard, E., N. Thierry-Mieg and S. Ricard-Blum, 2009. Interaction networks: From protein functions to drug discovery. A review. Pathol. Biol., 57: 324-333.

Cheng, F., C. Liu, J. Jiang, W. Lu and W. Li *et al.*, 2012. Prediction of drug-target interactions and drug repositioning via network-based inference. PLoS Comput. Biol., Vol. 8. 10.1371/journal.pcbi.1002503.

Chrysant, S.G., M.A. Weber, A.C. Wang and D.J. Hinman, 2004. Evaluation of antihypertensive therapy with the combination of olmesartan medoxomil and hydrochlorothiazide. Am. J. Hypertens., 17: 252-259.

Csermely, P., V. Agoston and S. Pongor, 2005. The efficiency of multi-target drugs: The network approach might help drug design. Trends. Pharmacol. Sci., 26: 178-182.

Dong, J. and S. Horvath, 2007. Understanding network concepts in modules. BMC Syst. Biol., Vol. 1. 10.1186%2F1752-0509-1-24.

Forbes, C.D., 1998. Secondary stroke prevention with low-dose aspirin, sustained release dipyridamole alone and in combination. ESPS Investigators. European Stroke Prevention Study. Thromb. Res., 92: S1-S6.

Ho, D.D., 1995. Time to hit HIV, early and hard. N. Engl. J. Med., 333: 450-451.

Hopkins, A.L., 2008. Network pharmacology: The next paradigm in drug discovery. Nat. Chem. Biol., 4: 682-690.

Jeong, H., S.P. Mason, A.L. Barabasi and Z.N. Oltvai, 2001. Lethality and centrality in protein networks. Nature, 411: 41-42.

Kairuz, T.E., D. Gargiulo, C. Bunt and S. Garg, 2007. Quality, safety and efficacy in the off-label use of medicines. Curr. Drug Saf., 2: 89-95.

Kerrien, S., B. Aranda, L. Breuza, A. Bridge and F. Broackes-Carter *et al.*, 2012. The IntAct molecular interaction database in 2012. Nucleic Acids Res., 40: D841-D846.

Knox, C., V. Law, T. Jewison, P. Liu and S. Ly *et al.*, 2011. DrugBank 3.0: A comprehensive resource for omics research on drugs. Nucleic Acids Res., 39: D1035-D1041.

Kotlyar, M., K. Fortney and I. Jurisica, 2012. Network-based characterization of drug-regulated genes, drug targets and toxicity. Methods, 57: 499-507.

Kroeze, W.K., K. Kristiansen and B.L. Roth, 2002. Molecular biology of serotonin receptors structure and function at the molecular level. Curr. Top. Med. Chem., 2: 507-528.

Lee, S., K. Park and D. Kim, 2009. Building a drug-target network and its applications. Exp. Opin. Drug Discov., 4: 1177-1189.

Marre, M. and T. Allavoine, 2000. Response rate in second-line therapy of a new fixed combination metformin/glibenclamide product. Diabetes Res. Clin. Pract., 50: 343-343.

Paolini, G.V., R.H. Shapland, W.P. van Hoorn, J.S. Mason and A.L. Hopkins, 2006. Global mapping of pharmacological space. Nat. Biotechnol., 24: 805-815.

Prasad, T.S.K., R. Goel, K. Kandasamy, S. Keerthikumar and S. Kumar *et al.*, 2009. Human protein reference database: 2009 update. Nucleic Acids Res., 37: D767-D772.

Raffa, R.B., R. Clark-Vetri, R.J. Tallarida and A.I. Wertheimer, 2003. Combination strategies for pain management. Exp. Opin. Pharmacother., 4: 1697-1708.

Roth, B.L., J.S. Douglas and K.K. Wesley, 2004. Magic shotguns versus magic bullets: Selectively non-selective drugs for mood disorders and schizophrenia. Nat. Rev. Drug Discov., 3: 353-359.

Ruffner, H., A. Bauer and T. Bouwmeester, 2007. Human protein-protein interaction networks and the value for drug discovery. Drug Discov. Today, 12: 709-716.

Salwinski, L., C.S. Miller, A.J. Smith, F.K. Pettit and J.U Bowie, D. Eisenberg, 2004. The database of interacting proteins. Nucleic Acids Res., 32: 449-451.

Scheffer, R.E., 2004. Abnormal neurological signs at the onset of psychosis. Schizophrenia Res., 70: 19-26.

Smoot, M.E., K. Ono, J. Ruscheinski, P.L. Wang and T. Ideker, 2011. Cytoscape 2.8: New features for data integration and network visualization. Bioinformatics, 27: 431-432.

Stark, C., B.J. Breitkreutz, A. Chatr-Aryamontri, L. Boucher and R. Oughtred *et al*., 2011. The BioGRID interaction database: 2011 update. Nucleic Acids Res., 39: D698-D704.

Sud'ina, G.F., M.A. Pushkareva, P. Shephard and T. Klein, 2008. Cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) selectivity of COX inhibitors. Prostaglandins Leukot. Essent. Fatty Acids, 78: 99-108.

Tao, W., X. Xu, X. Wang, B. Li, Y. Wang, Y. Li and L. Yang, 2013. Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal *Radix Curcumae* formula for application to cardiovascular disease. J. Ethnopharmacol., 145: 1-10.

Vogt, I. and J. Mestres, 2010. Drug-target networks. Mol. Inform., 29: 10-14.

Weber, A., A. Casini, A. Heine, D. Kuhn, C.T. Supuran, A. Scozzafava and G. Klebe, 2004. Unexpected nanomolar inhibition of carbonic anhydrase by COX-2-selective celecoxib: New pharmacological opportunities due to related binding site recognition. J. Med. Chem., 47: 550-557.

Xu, Z., 2011. One step at a time. Nature, 480: S90-S92.

Yamanishi, Y., M. Araki, A. Gutteridge, W. Honda and M. Kanehisa, 2008. Prediction of drug-target interaction networks from the integration of chemical and genomic spaces. Bioinformatics, 24: i232-i240.

Yildirim, M.A., K.I. Goh, M.E. Cusick, A.L. Barabasi and M. Vidal, 2007. Drug-target network. Nat. Biotechnol., 25: 1119-1126.